Amphetamine Maintenance Therapy During Intermittent Cocaine Self-Administration in Rats Attenuates Psychomotor and Dopamine Sensitization and Reduces Addiction-like Behavior

Supplemental Information

Supplemental Methods

Subjects

Male Wistar rats (200-250 g; Charles River Laboratories, St Constant, Qc) were housed 1/cage under a 12-h reverse light-dark cycle (lights off at 08:30 am). Upon arrival to the animal colony, rats were left undisturbed for 3 days with ad libitum access to food and water. After this, rats were handled daily during the dark phase of the circadian cycle and food was restricted [1] to 25 g/day with the following exceptions: 15 g/day during food self-administration training and 35 g on the days surrounding catheter implantation. Water was available *ad libitum*.

Food self-administration training

All self-administration training and testing took place in standard operant conditioning cages that also contained infrared photocells to measure horizontal locomotor activity. At the beginning of each session, the house-light was illuminated, and the levers were inserted. Pressing the active lever was reinforced with one banana-flavored food pellet (45 mg; grain-based; VWR, Montreal, QC) under a fixed ratio 1 schedule (FR1). Upon each reward delivery, the light above the active lever was turned on and both levers were retracted during a 20-s timeout period. After this timeout period, the light above the active lever was turned off and both levers were again inserted into the cage. Pressing the inactive lever had no programmed consequences. Each food training session lasted 1 h or until 100 food pellets were delivered. At the end of each session, the house-light was turned off and the levers were retracted. All rats met the acquisition criterion (taking ~100

pellets/session on two consecutive days) in 2-3 days. Next, for two sessions, food pellets were available under FR3. The i.v. catheters were then implanted into the jugular vein of the rats.

Catheter implantation

On the day following the end of food training, catheters were implanted into the jugular vein of the rats [2-4], under isofluorane anesthesia (5% for induction, 2-3% for maintenance). Homemade catheters consisted of a 12.5-cm length of silastic tubing linked to a stainless-steel cannula (C313G-5UP). Dental cement was then used to affix the cannula to a circular piece of nylon mesh. Two silicone bubbles were placed on the silastic tubing. These bubbles served to fasten the catheter to the jugular vein and to the chest muscle with suture thread. The cannula with the nylon mesh was set to lie subcutaneously in between the rats' scapulae. On the day of surgery, rats received an intramuscular injection of a penicillin antibiotic (Procillin; CDMV, St Hyacinthe, Qc) and a subcutaneous injection of the anti-inflammatory agent Carprofen (Rimadyl; CDMV, St Hyacinthe, Qc). To avoid coagulation, catheters were flushed each day with a sterile saline solution and every other day with a heparinized saline solution (0.2 mg/ml; Sigma-Aldrich, Oakville, ON).

Drugs

Cocaine hydrochloride (Medisca Pharmaceutique, St Laurent, Qc; 0.25 mg/kg/injection, delivered over 5 s) and D-amphetamine (A; Sigma-Aldrich, Dorset, UK) were dissolved in 0.9% saline. An osmotic minipump (Alzet model 2ML2, Durect, Cupertino, CA, USA) set to deliver 5 mg/kg/day D-amphetamine for 14 days was implanted in each COC + A or SAL + A rat. This dose and regimen of D-amphetamine administration was selected based on prior work in rats [5, 6] showing that it decreases responding for cocaine under a progressive ratio schedule of reinforcement, but it increases responding for sucrose pellets and does not reduce body weight, suggesting no debilitating or overtly toxic effects. Of note, D-amphetamine administration via minipump can

induce neurotoxicity in rats, as indicated by degenerating axon terminals or tyrosine hydroxylase immunoreactive patches, but at doses above 16-20 mg/kg/day [7-9]. We weighed the rats before minipump implantation and we then estimated body weight mid-way through the projected 14-day D-amphetamine treatment. Actual body weights on day 7 of D-amphetamine treatment showed that rats received ~5.1 mg/kg/day D-amphetamine.

Cocaine self-administration training

The rats were tested in operant conditioning cages (Med Associates, St Albans, VT) containing two retractable levers, a cue light above each lever and infrared photocells to measure horizontal locomotor activity. After acquisition of operant responding for food pellets, rats learned to self-administer cocaine (0.25 mg/kg/injection, delivered over 5 s) during 1-h sessions (1 session/day) under a fixed ratio 3 schedule of reinforcement (FR3). Each session started with illumination of the house light and insertion of two levers. Pressing the active lever produced a cocaine injection, illumination of a light cue above the active lever and retraction of both levers for a 20-s timeout period during which lever presses were not reinforced. The light cue was on for the 5-s injection and the ensuing 20-s time out period. Pressing the inactive lever had no consequences. We considered that rats acquired reliable cocaine self-administration behavior when on two consecutive days, they took ≥ 6 injections/session, at regular intervals throughout the session, and pressed ≥ 2 times more on the active versus inactive lever [10-12].

Osmotic minipump implantation

After acquiring cocaine self-administration, rats were anaesthetized with isofluorane and a minipump was inserted subcutaneously on the animals' backs, caudal to the catheter. Rats allocated to the D-amphetamine condition were implanted with a minipump filled with D-amphetamine. These are the COC + A rats in Experiment 1, and COC + A as well as SAL + A rats in Experiment 2. In Experiment 1, rats allocated to the COC group were implanted with a

minipump filled with saline or received a sham surgery consisting of an incision and sutures. In Experiment 2, COC and SAL rats received a sham surgery.

Effects of cocaine on dopamine uptake inhibition in the nucleus accumbens core (NAcC) *ex vivo*

We used a fast scan cyclic voltammetry (FSCV) protocol similar to that described in our previous work [13-15]. Rats were injected i.p. with sodium pentobarbital (90 mg/kg) and perfused with a NMDG-based solution (in mM; 92 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 30 $NaHCO_3$, 20 HEPES, 25 glucose, 2 thiourea, 5 ascorbic acid, 3 sodium pyruvate, 2 $CaCl_2$, 2 $MgSO_4$) for at least 1 hour at room temperature. The brain was extracted, and 300-µm-thick coronal slices were prepared in ice-cold (0 to 4°C) NMDG solution using a vibrating blade microtome (Leica VT1000S). Slices were first placed in a 32°C NMDG solution for 12 min and then in an oxygenated HEPES-buffered resting solution (in mM; 92 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 30 $NaHCO_3$, 20 HEPES, 25 glucose, 2 thiourea, 5 ascorbic acid, 3 sodium pyruvate, 2 $CaCl_2$, 2 $MgSO_4$) for at least 1 h at room temperature. A slice containing the NAcC (approximately +1.7 mm from Bregma [16]) was placed in the recording chamber and continuously perfused (1 ml/min) with oxygenated aCSF (in mM; 119 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 24 $NaHCO_3$, 12.5 glucose, 2.4 $CaCl_2$, 1.3 $MgSO_4$) at 32°C. The carbon-fiber electrodes (~7 µm in diameter) were selected for their sensitivity to dopamine using *in vitro* calibration with 1 µM dopamine in aCSF before each experiment and were placed ~100 µm below the surface into the NAcC and centered between the two poles of the bipolar stimulating electrode (placed on the surface of the slice; Plastics One, Roanoke, VA, US). Single-pulse electrical stimulations (400 µA; 1 ms) were generated every 5 minutes to evoke dopamine release and the potential at the carbon fiber electrode was scanned according to a 10-ms triangular voltage wave (-0.4 to 1 to -0.4 V vs. Ag/AgCl, at the rate of 300 V/s), using an Axon instrument, CV 203BU headstage preamp and an Axopatch 200B amplifier (maximum voltage range +/- 1V; Axon Instruments, Union City, CA). These parameters are well validated in the field and they are adapted for use with patch-clamp amplifiers [15, 17], as was done here. Data were

acquired using a Digidata 1440a analog to digital converter board (Axon Instruments) and Clampex software (Axon Instruments). Dopamine release was analyzed as the peak height of electrically-evoked extracellular dopamine overflow. Once three stable responses were recorded, increasing concentrations of cocaine (0.3, 1, 3, 10, 30 μM) were cumulatively applied to the bath. Once 3 stable responses were recorded at a given cocaine concentration, the next concentration was applied.

Analysis of the kinetics of fast-scan cyclic voltammetry data

Dopamine reuptake was modelled from the rate of recovery of the dopamine signal using Michaelis-Menten kinetics [18]. First, nonlinear least-square optimization based on the Levenberg-Marquardt method (scipy.optimize implementation in python) was applied to fit the three parameters 'a' (amplitude of the dopamine response), 'b' (dopamine concentration end value) and 'τ' of the exponential function $[DA](t) = a \cdot \exp(-t/\tau) + b$ to the reuptake phase of the dopamine response. The time constant τ (tau) of the exponential corresponds to the half-life divided by log(2) and is related to the Michaelis-Menten parameters Vmax and Km. The exponential approximation is more accurate when the amplitude of the dopamine signal is not too large relative to Km, as was the case in our experimental recordings. It can be shown by integration that the area under the exponential function and the area under an equivalent Michaelis-Menten curve are equal if and only if $\tau \cdot Vmax = Km + [DA]/2$, where [DA] is dopamine peak height. Under the assumptions that Km = 0.18 μM in cocaine-free conditions and Vmax remains constant during each experiment [19], this formula provides an estimate of Km (apparent Km, or app. Km) based on τ and [DA], while avoiding overfitting. The parameters [DA] and app. Km were extracted from each recording. In cocaine-free conditions, signals were of relatively low amplitude, such that – across experimental groups – signal-to-noise ratio was sometimes suboptimal for accurate parameter identification (**Figure S4**). As such, we computed kinetic

parameters as percentages relative to 0.3 μM cocaine. As illustrated in **Figure S4**, this concentration did not have a large effect on dopamine uptake inhibition. Indeed, in each of our four groups, app. Km values at 0.3 μM cocaine and baseline were similar (two-tailed, one-sample *t*-tests compared to the mean 0.18 μM; All *P*'s > 0.1). A one-sample test can possibly fail to detect small potential differences compared to a paired *t*-test. However, consistent with our observations, in Calipari et al (2013), app. Km at 0.3 μM cocaine was only < 10-20% greater than control levels (see their Figure 3C; albeit no formal statistical comparisons were reported), which remains small compared to the increases evoked by higher cocaine concentrations (> 1000%; Also see [18, 20]).

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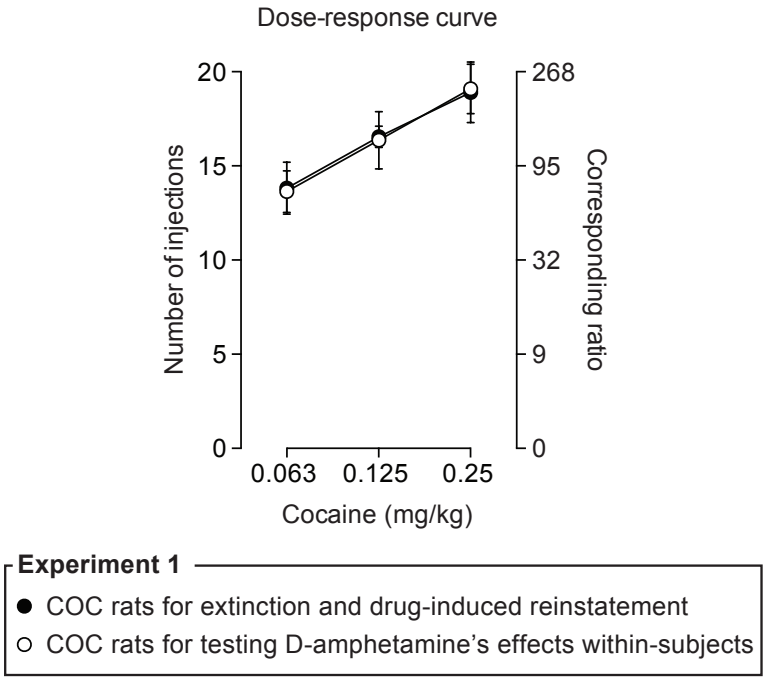

Figure S1. Responding for cocaine under a progressive ratio schedule of reinforcement in COC rats used in Experiment 1. In Experiment 1, after being tested for responding for cocaine (0.063-0.25 mg/kg/infusion) under a progressive ratio schedule of reinforcement, the 22 COC rats were divided into two groups. One group was then tested for extinction and cocaine-induced reinstatement of cocaine-seeking behavior. The other group was given additional IntA-sessions to self-administer cocaine, but now with D-amphetamine treatment. The figure shows that motivation to take cocaine was comparable in the two groups before allocation to subgroups. Data are mean ± SEM. n = 11/group. IntA, Intermittent Access. COC, Cocaine. A, D-amphetamine

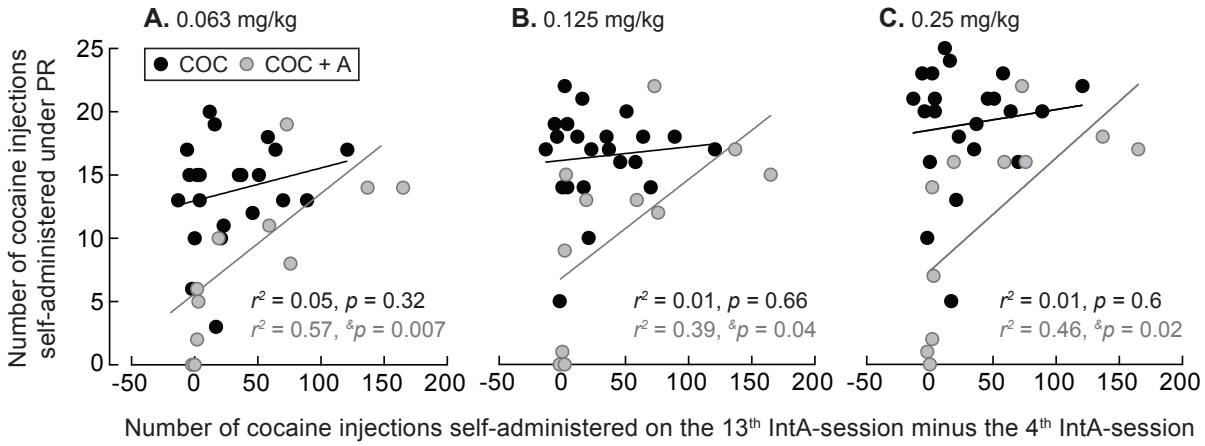

Figure S2. Escalation of cocaine intake during IntA-sessions predicted responding for cocaine under a progressive ratio schedule in COC + A rats, but not in COC rats. Correlations between the difference in number of cocaine injections taken during IntA-sessions 13 and 4 (an index of escalation) and the number of injections later taken under progressive ratio (PR) at 0.063 mg/kg/infusion (**A**), 0.125 mg/kg/infusion (**B**) and 0.25 mg/kg/infusion (**C**) cocaine. &p < 0.05, non-zero slope.

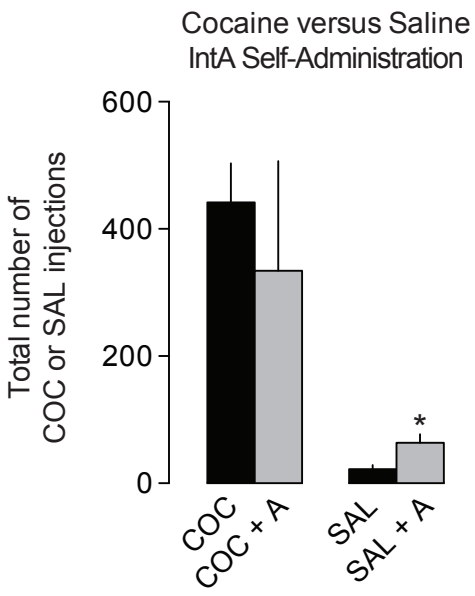

Figure S3. D-amphetamine maintenance did not significantly change the total number of self-administered cocaine injections over the 14 intermittent-access sessions. D-amphetamine increased inter-individual variability in cocaine intake, but average COC intake was equivalent with or without D-amphetamine maintenance. D-amphetamine maintenance increased the number of self-administered saline injections. *p = 0.02, versus SAL + A. IntA, Intermittent Access. COC, Cocaine. SAL, Saline. A, D-amphetamine.

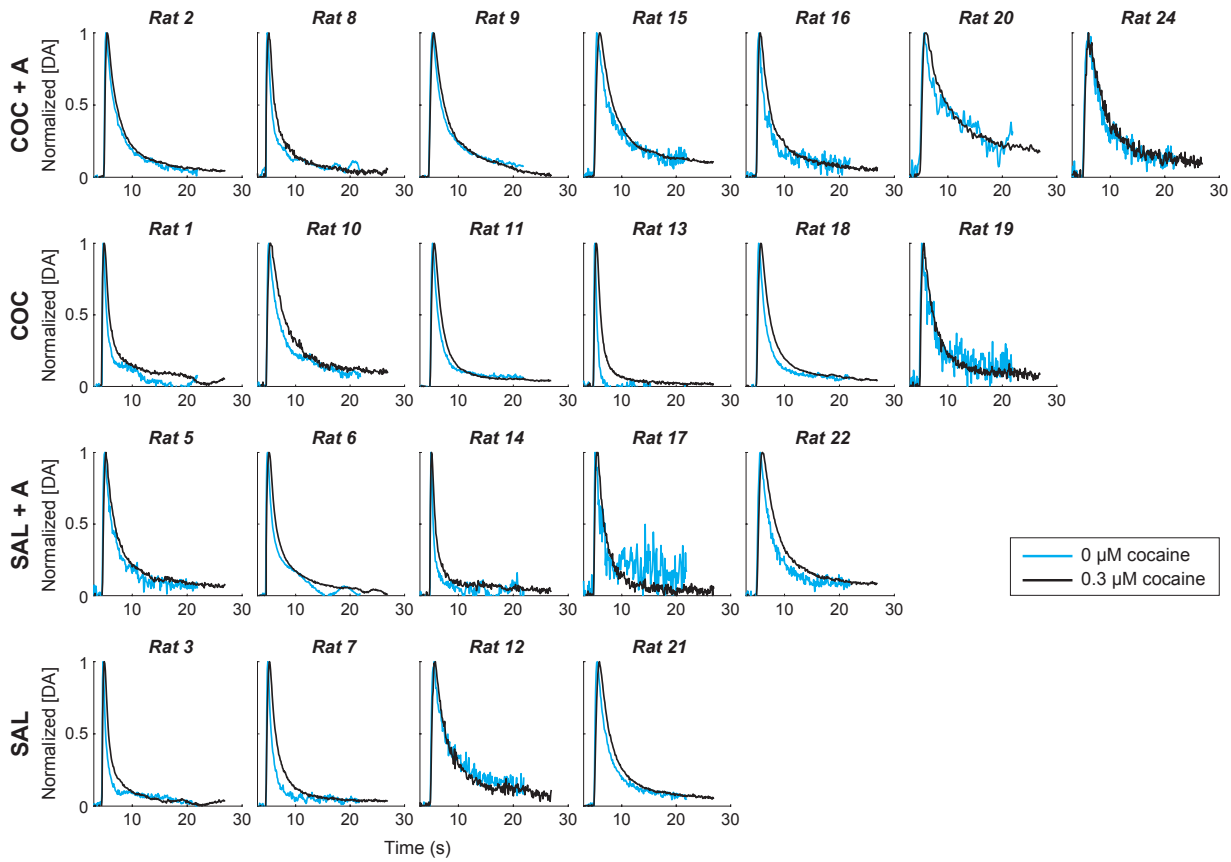

Figure S4. Fast-scan cyclic voltammetry traces in individual rats at baseline (0 µM) and after bath application of 0.3 µM cocaine. At 0 µM cocaine, signals were of relatively low amplitude, and signal-to-noise ratio was sometimes suboptimal for accurate parameter identification. Baseline traces with low signal-to-noise ratio were distributed across experimental groups, and not biased to specific groups. Compared to baseline, noise was lower at 0.3 µM cocaine, and this concentration did not have a large effect on dopamine uptake inhibition. COC, Cocaine. SAL, Saline. A, D-amphetamine.

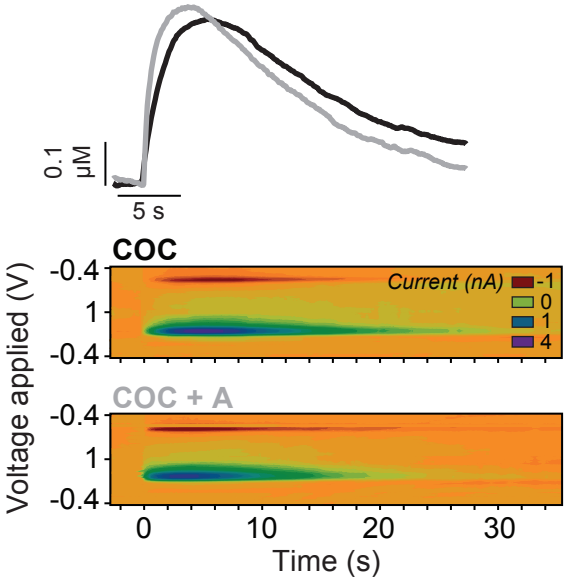

Figure S5. Representative traces (top) and pseudo-color plots (bottom) in COC and COC + A rats following bath application of 30 µM cocaine. Pseudo-color plots illustrate a prolonged signal in COC rats compared to COC + A rats. These rats were selected based on their similar DA peak heights. COC, Cocaine. A, D-amphetamine.